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Title: Changes in Clavicle Length and Maturation in Americans: 1840-1980

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Key words: secular change, skeletal maturation, clavicle, epiphyseal union, clavicle length, LOESS regression, piecewise regression

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ABSTRACT

Secular changes refer to short-term biological changes ostensibly due to environmental factors. Two well-documented secular trends in many populations are earlier age of menarche and increasing stature. This study synthesizes data on maximum clavicle length and fusion of the medial epiphysis in 1840-1980 American birth cohorts to provide a comprehensive assessment of developmental and morphological change in the clavicle.

Clavicles from the Hamann-Todd Osteological Collection (n=354), McKern and Stewart Korean War males (n=341), Forensic Anthropology Data Bank (n=1239), and University of Tennessee McCormick Clavicle Collection (n=1137) were used in the analysis. Transition analysis was used to evaluate fusion of the medial epiphysis (scored as unfused, fusing, or fused). Several statistical treatments were used to assess fluctuations in maximum clavicle length. First, Durbin-Watson tests were used to evaluate autocorrelation, and a LOESS regression (local regression) was used to determine visually if any observable shifts in the regression slope exist. Next, piecewise regression was used to fit linear regression models before and after the estimated breakpoints. Multiple starting parameters were tested in the range determined to contain the breakpoint, and the model with the smallest mean squared error was chosen as the best fit. The parameters from the best fit models were then used to derive the piecewise models, which were compared to the initial simple linear regression models to determine which model provided the best fit for the secular change data.

The epiphyseal union data indicates a decline in the age-at-onset of fusion since the early 20th century. Fusion commences approximately four years earlier in mid to late 20th century birth cohorts compared to late 19th and early 20th century birth cohorts. However,

fusion is completed at roughly the same age across cohorts. The most significant decline in age-at-onset of epiphyseal union appears to have occurred since the mid-twentieth century. LOESS regression plots show a breakpoint in the clavicle length data around the mid-twentieth century in both sexes, and piecewise regression models indicate a significant decrease in clavicle length in the American population after 1940. The piecewise model provides a slightly better fit than the simple linear model. Since the model standard error is not substantially different from the piecewise model, an argument could be made to select the less complex linear model. However, we chose the piecewise model to detect changes in clavicle length that are over fitted with a linear model.

The decrease in maximum clavicle length is in line with a documented narrowing of the American skeletal form, as shown by analyses of cranial and facial breadth and bi-iliac breadth of the pelvis. Environmental influences on skeletal form include increases in body mass index, health improvements, improved socioeconomic status, and elimination of infectious diseases. Secular changes in bony dimensions and skeletal maturation stipulate that medical and forensic standards used to deduce information about growth, health, and biological traits must be derived from modern populations.

Key words: secular change, skeletal maturation, clavicle, epiphyseal union, clavicle length, LOESS regression, piecewise regression

Human biologists use the terms “secular trends” and “secular changes” to refer to biological changes that occur over several decades or generations ostensibly due to environmental factors (Roche 1979). According to Malina (1979), secular changes are not caused necessarily by introducing growth-stimulation factors, but rather by eliminating growth-inhibiting factors (i.e. nutritional stress, environmental stresses, and disease). Improvements in socioeconomic status, nutrition, and health care in the US population at large over the past century have contributed to increases in weight and stature, as well as accelerated maturation. Stature increases have been documented in populations worldwide using anthropometric and osteometric datasets (e.g. Blanksby 1995, Buretic-Tomljanovic et al. 2004, Chen and Ji 2014, Fredriks et al. 2000, Freedman et al. 2000, Meadows Jantz and Jantz 1999, Meadows and Jantz 1995, Meredith 1976, Ulijaszek 1993). Stature is a polygenic trait and, consequently, is influenced by environmental fluctuations, but populations (and individuals) have a genetic potential to attain a certain stature. This is reflected in a study on secular change in stature of American-born Mexican children (Malina et al. 1987). American-born generations were taller than their Mexican-born ancestors, but Mexican Americans were shorter than American whites and blacks. The authors attribute shorter stature in Mexican populations to lower socioeconomic status *and* ethnic variation in body size. As Relethford (2004) explains, individuals who immigrate to a different population will experience a certain amount of change due to natural selection, developmental plasticity, and gene flow, but these changes are not enough to erase the underlying patterns of population history and structure.

The most extensively documented positive secular trend in maturation is decreasing menarcheal age, which has been studied for more than a century in populations around the

world. Malina (1979) documented secular changes in menarcheal age in Europe before, during, and after the Industrial Revolution. Disease, nutritional, and social stresses associated with overcrowded cities during the Industrial Revolution initially caused a delay in maturation relative to pre-industrial rates. Post-industrial Revolution improvements in environmental and nutritional quality led to accelerated growth. Since then Europeans have experienced rather stable caloric intakes and reduced caloric expenditures and, consequently, increases in stature and body weight. A brief negative secular trend in menarcheal age occurred during World War II, but this was minor and temporary, and acceleration resumed once social and economic conditions were restored to pre-war levels. The Industrial Revolution and WWII effects on maturation illustrate the remarkable plasticity of this aspect of human biology. Generally, age at menarche in western industrialized populations has decreased over the past 4-5 decades by around 4-6 months per decade (Bagga and Kulkarni 2000, Fredriks et al. 2000, Hoshi and Kouchi 1981, Huen et al. 1997, Hwang et al. 2003, La Rocherbrochard 2000, Liu et al. 2000, Rimpela and Rimpela 1993). Although the precise age of pubertal onset is more difficult to detect in males, a marked decrease in the age of voice breaking in males has been documented (La Rocherbrochard 2000). Accelerated skeletal maturation has also been reported in the hand and wrist epiphyses (Himes 1984, So and Yen 190), tibia and fibula (Crowder and Austin 2005), and medial clavicle (Langley-Shirley and Jantz 2010). While it has been proposed traditionally that dental development is under stricter genetic control than epiphyseal union and less susceptible to environmental fluctuations, secular changes have been observed in dental maturation (Bernhard 1995, Cardoso et al. 2010, Jayaraman et al. 2013).

This analysis synthesizes the data on secular changes in clavicle length and fusion timing of the medial epiphysis in the American population from 1840-1980 birth cohorts to provide a comprehensive assessment of developmental and morphological change in the clavicle.

Langley-Shirley and Jantz (2010) found that union of the medial/sternal epiphysis commenced significantly earlier in late 20th century birth cohorts compared to early 20th century cohorts. In light of the documented increases in long bone length and accelerated maturation in the lower limb (Crowder and Austin 2005, Meadows and Jantz 1995), we hypothesize that clavicle length has also increased.

MATERIALS AND METHODS

The sample consists of clavicles from the Hamann-Todd Osteological Collection (n=354), McKern and Stewart Korean War males (n=341), the Forensic Anthropology Data Bank (n=1239), and the McCormick Clavicle Collection at the University of Tennessee (n=1137). The Hamann-Todd Human Osteological Collection is housed at the Cleveland Museum of Natural History. The collection consists of skeletal remains from approximately 3,000 cadavers autopsied or used for medical school dissection between 1912 and 1938. The demographic composition of the Hamann-Todd Collection is 62% European American and 38% African American. The McKern and Stewart data was collected from 1950-1952 Korean War fatalities as part of a report to the US Government entitled *Skeletal Age Changes in Young American Males* (1957). The McKern and Stewart sample is 87% European American and 11% African American, and 2% other (Mexican, Puerto Rican, or Native American). This data is available online (<http://konig.la.utk.edu/paleod.htm>), and Stewart's original observations are maintained in the National Anthropological Archives at the Smithsonian. The Forensic Anthropology Data Bank

(FDB) contains osteological and demographic information from forensic cases, including date and place of birth, medical history, occupation, stature, and weight. The FDB has nearly 3400 cases, approximately 2400 with known sex and ancestry. The FDB also has osteometric data from the Robert J. Terry Anatomical Collection at the Smithsonian Institution in Washington, D.C. The demographics of the FDB sample used in this analysis is 87% European American and 13% African American. The William F. McCormick clavicles at the University of Tennessee are a documented autopsy sample of approximately 2,000 clavicle pairs from 1986-1998 autopsies in East Tennessee. The demography of the sample is 95% European American, 4% African American, and 1% other (Latino, Asian, and Native American). Epiphyseal union data was obtained from the Hamann-Todd, McKern and Stewart, and McCormick samples; length data was collected for the McCormick and Forensic Data Bank clavicles. The composition of both analytical samples (maximum length and epiphyseal union) is provided in Table 1.

Transition analysis was used to evaluate fusion of the medial epiphysis (Boldsen et al. 2002). Details of this analysis are available in Langley-Shirley and Jantz (2010), but the analytical methods are summarized briefly for the purposes of the present analysis. The epiphysis was scored as unfused (epiphysis not united to the shaft), fusing (epiphysis in the process of union), or fused (epiphysis completely united to the shaft). A cumulative probit model on log age option (proportional odds model with a probit link) was performed in Konigsberg's Nphases program (available at <http://konig.la.utk.edu/nphases2.htm>). The Fortran-based program performs a logistic regression where the intercept and slope are converted to the mean and standard deviation, respectively (Boldsen et al. 2002). Nphases gives an age at transition, which is the maximum likelihood estimate of the likelihood function provided by the transition

analysis. This estimate represents the average age at which an individual is most likely to transition from one phase to the next. Males and females were analyzed separately, and a preliminary separate analysis of blacks and whites was performed to test for ethnic differences in skeletal maturation. The maximum likelihood estimates of the transitions and corresponding standard errors were used to calculate student's t-statistics to evaluate differences in the transition ages between ethnicities, sexes, and birth cohorts.

A combination of statistical treatments was used to assess fluctuations in maximum clavicle length. First, Durbin-Watson tests were used to evaluate autocorrelation in the dataset in order to determine which type of regressive model could be employed to analyze the data. A simple linear regression was run on the male and female datasets to test the appropriateness of using a linear model for accounting for the variation in clavicle length with time. Additionally, LOESS regression (local regression) was used as an exploratory method to determine if the data deviates from a linear model. The LOESS method fits multiple regression models to localized subsets of the data and is a more sensitive method for detecting subtle fluctuations in the data, as it does not assume that the relationship between independent and dependent variable is linear. The results of the LOESS plots were used to determine visually if any breakpoints exist in the data. Breakpoints, or change points, are points where an observable shift in the regression slope occurs. Simple linear and LOESS regression analyses were executed with NCSS software (2015).

Next, a combination of two linear regression models were fit before and after the estimated breakpoints in the data for both male and female datasets in order to obtain starting parameters for the piecewise regression model. This technique is referred to as piecewise

regression, and it allows for multiple linear models to be fit to the data for different ranges. Starting parameters required for the piecewise models were obtained by first estimating a simple linear model above and below each estimated breakpoint. Multiple starting parameters were tested in the range determined to contain the breakpoint, and the model with the smallest mean squared error was chosen as the best fit. The parameters from the best fit models were then used to derive the piecewise models, which were compared to the initial simple linear regression models to determine which model provided the best fit for the secular change data. The final piecewise regression models were evaluated for convergence, Q-Q (quantile-quantile) plots were used to examine the normality of the residuals, and the Wald Z-test was used to test the residuals for autocorrelation. The piecewise regression analyses were done in SAS 9.2 (SAS Institute, Inc., 2008).

RESULTS

The epiphyseal union data indicate a decline in the age-at-onset of fusion since the early 20th century. A Student's t-test with a 95% confidence interval revealed no significant differences in the transition ages between European Americans and African Americans in the male or female sample, so ethnicities were combined. Table 2 shows the results of the transition analysis. Fusion commences approximately 4 years earlier in mid to late 20th century birth cohorts represented by the McCormick individuals (1955-1985) compared to late 19th and early 20th century birth cohorts (1880-1935) represented by the Hamann-Todd and McKern and Stewart individuals. The t-statistics revealed significant differences in fusion commencement between McCormick individuals and each of the late 19th/early 20th century samples ($p < .05$). However, fusion is completed at roughly the same age across cohorts. The most drastic decline

in age-at-onset of epiphyseal union appears to have occurred since the mid-twentieth century. Sexual dimorphism was more pronounced in the Hamann-Todd sample ($p < .10$) than in the McCormick sample ($p > .10$).

Figures 1 and 2 show the LOESS regression plots for males and females, respectively. Both sexes experience an overall increase in maximum clavicle length from approximately the mid-nineteenth to the mid-twentieth century, and a decrease thereafter. Durbin-Watson tests did not detect positive or negative autocorrelation in the data ($p > .05$). Figures 3 and 4 provide a graphic representation of the linear models and breakpoints investigated in the piecewise regression for the male and female samples. Two piecewise regression models were employed for each group to test subsets of the larger dataset for significant changes in clavicle length before and after the breakpoint with the lowest mean squared error (birth year = 1940). Each data subset began or ended with the estimated breakpoint. The linear regression models applied to the male and female data subsets before and after the 1940 breakpoint are presented in Tables 3 and 4. These models provided the best fit for the data, therefore the parameters from these models were used as starting parameters for the piecewise model. Tables 5 and 6 compare the fit of the linear regression versus piecewise regression models for the male and female datasets. Based on comparisons of the model standard error and on the visual fit (see Figures 1 and 2), the piecewise linear model provides a slightly better fit than the simple linear model. Since the model standard error is not substantially different from the piecewise model, an argument could be made to select the less complex linear model. However, we choose the piecewise model to detect changes in clavicle length that are over fitted with a linear model.

Finally, the assumptions of the piecewise regression model were checked (convergence, independence of residuals, and normality of residuals). Convergence was met for the piecewise regression models, indicating that these models provided best fit for the subsets above and below the breakpoint. Next, the residuals were checked for independence. Wald Z-test results indicate that the residuals are significantly correlated (Table 7). However, since the mean square error values of the residuals (male model=76.5; female=71.5) are not appreciably different from those in the piecewise model (male=76.7, female=71.8), we assume the effects of the autocorrelation are not substantial. Q-Q plots indicated that the residuals were normal before and after the change point in both samples (males and females). Kolmogorov-Smirnov test statistics supported the Q-Q plot analysis except in the female dataset after the change point, which deviates from normality (Table 8).

DISCUSSION

This study presents an alternative way of analyzing temporal fluctuations in datasets. Durbin-Watson tests evaluate the presence of autocorrelation in the dataset, and LOESS regression plots provide a means of determining if the data deviates from a linear model. Running a simple linear regression also tests the degree to which a linear model accounts for variation in the dependent variable. If the data are not auto-correlated and deviate from a linear model, then piecewise regression provides an effective method for locating change points in the data, as demonstrated in this analysis.

Overall trends in maximum clavicle length between 1840 - 1980 birth cohorts are consistent among the sexes. Clavicle length in the American population increases until circa 1940 birth cohorts and then decreases significantly. One limitation of this analysis is that the

sample size for the 1840-1900 birth cohorts was smaller than for later birth cohorts.

Nonetheless, the change point in the data was detected around 1940, and the sample size before and after the change point was more than adequate for the statistical analyses.

The decrease in maximum length of the clavicle is counter to the changes documented in other long bones, which have increased in length over the past century in the American population (Meadows Jantz and Jantz 1999, Meadows and Jantz 1995). Yet the cranial vault and facial skeleton are also narrowing, as indicated by smaller values of bizygomatic breadth and maximum cranial breadth during the 125 years between 1850 and 1975 (Jantz 2001). One might surmise that the narrowing of the vault and shortening of the clavicle could be due in part to similarities in the embryological origins and ossification of these skeletal elements. Most skull bones are formed via intramembranous ossification, as is the lateral portion of the clavicle. Thus, these bones are derived from neural crest, whereas other long bones are derived solely from lateral plate mesoderm. However, bi-iliac breadth has also decreased (Delprete 2006, Driscoll 2010), and it is derived from lateral plate mesoderm, as well, so the decrease in cranial breadth and clavicle length cannot be attributed to a differential response of embryological germ tissues (i.e. neural crest versus lateral plate mesoderm). Instead, it appears that the human skeletal form is becoming more linear (longer and narrower), at least in the American population.

The epiphyseal union data indicates a shift towards earlier onset of fusion during the mid-1900s, as well. Unfortunately, a gap exists in the epiphyseal union data between 1934 and 1954, but the shift toward earlier onset of union began during this period, roughly at the same time as the significant decrease in maximum clavicle length. While pinpointing a particular

cause is difficult, it is worth noting that average BMI in Americans began to increase at the same time and has escalated most significantly during the past 50 years (Cutler et al. 2003). Increased BMI is related to reduced physical activity, which could have contributed to the reduction in clavicle length during the mid-twentieth century. Earlier onset of maturity also may be linked to higher BMI, as well as to the increased consumption of processed foods with added vitamins, nutrients, and hormones. Other factors contributing to body weight increases, overall health improvements, and accelerated maturation are: increased calcium intake, introducing cereals at early age in infant diet, increased consumption of processed sugars and fats, improved socioeconomic status, improved health status, improvements in water and sanitation, elimination of infectious diseases, reduction in infant mortality, increased life expectancy, and reduction in family size (Malina 1979).

It is interesting that fusion commences significantly earlier in modern cohorts of both sexes compared to earlier birth cohorts, but fusion is completed at roughly the same time regardless of birth year. This would appear ultimately to lengthen the time allotted for skeletal maturation, meaning that a change in the rate and intensity would be expected. One possibility for this discrepancy between onset and completion is that there is a constraint on when fusion is completed. Perhaps separate forces are driving each of these events: onset of fusion may be more sensitive to environmental influences, and completion may be under stricter genetic control. This conclusion cannot be deduced from the present analysis, but the hypothesis is worth mentioning given the results.

Secular change in bony dimensions and skeletal maturation stipulate that standards used by the medical and forensic communities to deduce information about growth, health,

and biological traits must be derived from modern populations. While population differences in skeletal form are present at or before birth, and environmental influences cannot erase the deep-seated genetic differences between populations, documented secular changes suggest a need for further evaluation of skeletal morphology in varying environments (Kouchi 2004).

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Table 1. Sample Composition.

| | Total n | Males | Females | European American | African American | Other (Hispanic, Asian, Native American) | Ages (in years) |
|-------------------------------|---------|---------------|--------------|----------------------|---------------------|--|--|
| Maximum Clavicle Length | 2374 | 1680 (71%) | 694 (29%) | 2054 (86.5%) | 315 (13.3%) | 5 (0.2%) | Range=18-101 Mean=51 SD=17.3 |
| Medial Epiphysis | 1289 | 1044 (71%) | 245 (19%) | 1129 (88%) | 146 (11%) | 14 (1%) | Range=11-33 Mean=22.6 SD=4.6 |

Table 2. Transition ages for the medial clavicle epiphysis (in years). Each age represents the maximum likelihood estimate of the transition from one phase to the next. S.D. = the standard deviation of the transitions.

| | Hamann-Todd Males | Hamann-Todd Females | McKern & Stewart (Males) | McCormick Males | McCormick Females |
|----------------------------|------------------------------|--------------------------------|---|----------------------------|------------------------------|
| Birth Cohort → | 1882-1927 | | 1917-1934 | 1954-1985 | |
| Transition ↓ | | | | | |
| Unfused- Fusing | 20.5 | 19.2 | 19.7 | 16.2 | 15.4 |
| Fusing-Fused | 27.9 | 25.4 | 26.0 | 26.0 | 25.4 |
| S.D. | 3.1 | 3.1 | 3.1 | 3.0 | 3.0 |

Table 3. Linear regression models applied above and below 1940 breakpoint for the male dataset.

| Linear Fit Below Estimated Breakpoint = 1940 (n=767) | | | | | |
|---|----|--------------------|----------------|---------|--------|
| Variable | DF | Parameter Estimate | Standard Error | t-value | Pr>t |
| Intercept | 1 | 48.335 | 37.755 | 1.28 | 0.201 |
| Year of Birth | 1 | 0.0574 | 0.0196 | 2.93 | 0.0035 |
| Linear Fit At and Above Estimated Breakpoint = 1940 (n=949) | | | | | |
| Variable | DF | Parameter Estimate | Standard Error | t-value | Pr>t |
| Intercept | 1 | 328.661 | 69.938 | 4.70 | <.0001 |
| Year of Birth | 1 | -0.0871 | 0.0358 | -2.43 | 0.0152 |

Table 4. Linear regression models applied above and below 1940 breakpoint for the female dataset.

| Linear Fit Below Estimated Breakpoint = 1940 (n=312) | | | | | |
|---|----|--------------------|----------------|---------|--------|
| Variable | DF | Parameter Estimate | Standard Error | t-value | Pr>t |
| Intercept | 1 | -34.048 | 45.234 | -0.75 | 0.452 |
| Year of Birth | 1 | 0.0915 | 0.0236 | 3.00 | 0.0001 |
| Linear Fit At and Above Estimated Breakpoint = 1940 (n=389) | | | | | |
| Variable | DF | Parameter Estimate | Standard Error | t-value | Pr>t |
| Intercept | 1 | 404.870 | 97.511 | 4.15 | <.0001 |
| Year of Birth | 1 | -0.135 | 0.0499 | -2.70 | 0.0072 |

Table 5. Linear versus Piecewise Regression Models for the Male Dataset.

| Piecewise Regression Model (Breakpoint = 1940) | | | | | |
|---|------|----------------|-------------|-----------------|--------|
| Source | DF | Sum of Squares | Mean Square | Approx. F Value | Pr>F |
| Model | 3 | 1081.3 | 360.4 | 4.70 | 0.0028 |
| Error | 1677 | 128590 | 76.7 | | |
| Linear Regression Model | | | | | |
| Source | DF | Sum of Squares | Mean Square | F-Ratio | Pr>F |
| Intercept | 1 | 421954.8 | 421954.8 | | |
| Slope | 1 | 0.04005 | 0.04005 | 0.0518 | 0.820 |
| Error | 1678 | 1293.94 | 77.3 | | |

Table 6. Linear versus Piecewise Regression Models for the Female Dataset.

| Piecewise Regression Model (Breakpoint = 1940) | | | | | |
|---|-----|----------------|-------------|-----------------|--------|
| Source | DF | Sum of Squares | Mean Square | Approx. F Value | Pr>F |
| Model | 3 | 1516.9 | 505.6 | 7.05 | 0.0001 |
| Error | 691 | 49590.5 | 71.8 | | |
| Linear Regression Model | | | | | |
| Source | DF | Sum of Squares | Mean Square | F-Ratio | Pr>F |
| Intercept | 1 | 138921.6 | 138921.6 | | |
| Slope | 1 | 1.253 | 1.253 | 1.72 | 0.190 |
| Error | 692 | 505.567 | 72.9 | | |

Table 7. Wald Z-tests for independence of the residuals.

| Male Dataset | | | | | | | | |
|----------------------|---------------|-------------------|--------------|-------|--------|-------|---------|--------|
| Covariance Parameter | Subject | Standard Estimate | Wald Z Error | Value | Pr(Z) | Alpha | Lower | Upper |
| AR(1) | Year of Birth | -0.0157 | 0.0252 | -0.62 | 0.533 | 0.05 | -0.0652 | 0.0337 |
| Residual | | 76.541 | 2.642 | 28.98 | <.0001 | 0.05 | 71.616 | 81.994 |
| Female Dataset | | | | | | | | |
| Covariance Parameter | Subject | Standard Estimate | Wald Z Error | Value | PrZ | Alpha | Lower | Upper |
| AR(1) | Year of Birth | 0.01300 | 0.0417 | 0.031 | 0.755 | 0.05 | -0.0687 | 0.0947 |
| Residual | | 71.459 | 3.837 | 18.62 | <.0001 | 0.05 | 64.497 | 79.618 |

Table 8. Kolmogorov-Smirnov tests of normality of the residuals.

| Test of Normality Below Breakpoint | | | |
|------------------------------------|-------|----------------------|--------|
| MALE DATASET | | FEMALE DATASET | |
| Kolmogorov-Smirnov D | Pr>D | Kolmogorov-Smirnov D | Pr>D |
| 0.0199 | 0.150 | 0.0396 | 0.150 |
| Test of Normality Above Breakpoint | | | |
| MALE DATASET | | FEMALE DATASET | |
| Kolmogorov-Smirnov D | Pr>D | Kolmogorov-Smirnov D | Pr>D |
| 0.0202 | 0.150 | 0.0589 | <.0100 |

Figure Captions

Figure 1. LOESS regression plot of length on birth year for the male sample. The horizontal straight line is fitted to a linear model, and the curved line is fitted to the LOESS model.

Figure 2. LOESS regression plot of length on birth year for the female sample. The horizontal straight line is fitted to a linear model, and the curved line is fitted to the LOESS model.

Figure 3. Linear models and breakpoint investigated in the male sample.

Figure 4. Linear models and breakpoint investigated in the female sample.

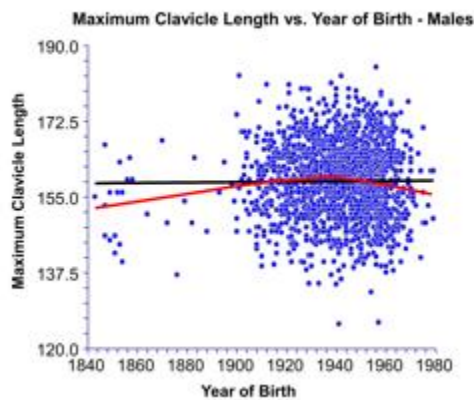


Figure 1.

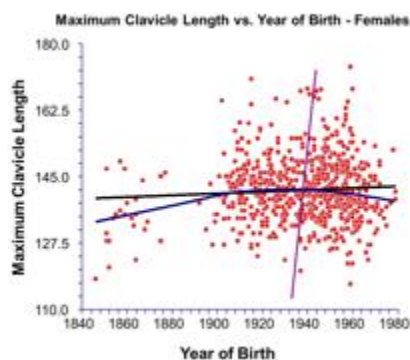


Figure 2.

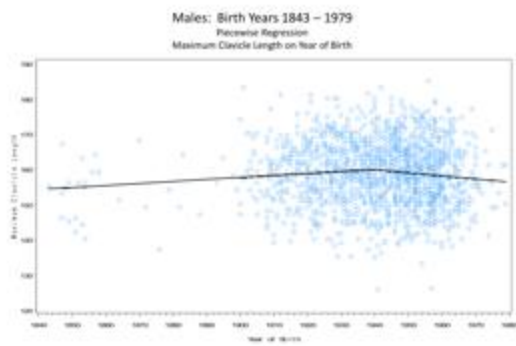


Figure 3.

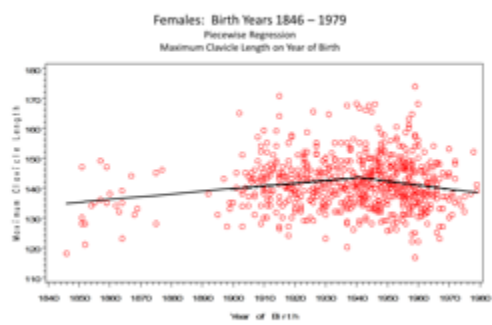


Figure 4.